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FRANK R. LILLIE,
Managing Editor.

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BIOLOGICAL BULLETIN

ON THE GEOTROPISM OF PARAMECIUM AND SPIROSTOMUM.[1]

SAKYO KANDA,

DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF MINNESOTA.

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I. INTRODUCTION.

Through the kindness of Professor Ralph S. Lillie, I was given an opportunity to study in the physiological department of the Marine Biological Laboratory at Woods Hole, Mass., during the summer—July 1–September 4—of 1912. During my stay the following experiments were made under the direction of the instructors in the physiology course, especially of Professor E. P. Lyon. I wish here to acknowledge my indebtedness to Professors Garrey, Knowlton, Lillie, Lyon and Meigs.

The general phenomena of geotropism in the protozoa are well known through the work of Schwarz (22), Aderhold (1), Massart (18), Jensen (11–12), Sosnowski (23), Moore (19), Jennings (5–10), Lyon (16–17), Harper (3–4), and others. The description of the phenomena given in these papers is exhaustive and no recapitulation is needed.

In this paper an attempt has been made to subject to a critical

[1] From the Marine Biological Laboratory, Woods Hole, Mass.

and experimental reëxamination the various theories of geotropism in the protozoa, especially in *Paramecium* and *Spirostomum*. The experimental work has been largely confined, with variations, to the repetition of the work of Lyon and Harper.

As is well known, paramecia are extremely sensitive to chemicals or food, to contact stimuli, to water currents, to mechanical agitation, to changes of temperature, etc. For geotropic experiments, therefore, the sources of those stimuli must be carefully excluded. If this has been done and if paramecia washed in pure distilled water are placed in a vertical *clean* glass tube, they swim upward and gather at the top. In other words, paramecia under proper conditions are negatively geotropic. But Sosnowski (21, p. 131), Platt (20, p. 32), Jennings (9, p. 473; 10, p. 76), Lyon (17, p. 421), Harper (3, p. 995) and others have found considerable variation in the behavior of individuals in different cultures; some orient themselves downward and swim in that direction, while others are apparently indifferent to gravity. Nevertheless, in the majority of cases there is no doubt that gravity is the real "directive force." We are thus confronted by the established fact of negative geotropism in paramecia, but different investigators offer varying explanations for the phenomenon.

II. The Mechanical Theory.

From his study of the reactions of *Euglena* and *Chlamydomonas* to gravity, Schwarz in 1884 reached the conclusion that the negative geotropism of these animals is due to the direct influence of gravity on the organisms, inciting movement in the opposite direction (22). Aderhold in 1888 investigated *Euglena* (1, pp. 317–320) and *Desmid* (1, pp. 339–340), and came to the same conclusion as Schwarz.

Though he seems now to accept the "statolith theory" (25, pp. 526–527), Verworn claimed at first that the effect of the force of gravity exerted upon the protozoa is "purely physical" (24, p. 121). According to him, "the protoplasmic mass of greater specific gravity sinks toward the bottom . . . , while that of less specific gravity rises toward the surface and stays

in that position." On such a "purely physical ground," he criticized Aderhold and believed it self-evident that "in complete quiescence of the flagellum the posterior end of the protist should be directed downward and not the flagellated anterior end." "With its flagellum directed upward and moving it, such an oriented individual must move toward the surface of the water" (24, p. 122). Further, Verworn believed that the force of gravity does not act upon the organism as a stimulus. This view has been called by Davenport the "mechanical theory" (2, p. 121).

After experiments on bacteria, flagellata, and ciliata, Massart in 1891 arrived at the same conclusion as Schwarz and Aderhold; and he stated that his "experiments do not agree with Verworn's theory" (18, p. 166).

After a more extensive and careful study than any of his predecessors had made, Jensen in 1893 "disproved" the mechanical theory of Verworn and proposed his pressure theory instead. But Jensen's experiments were not beyond criticism for his methods were unsatisfactory. He "killed" 50 paramecia in a "filtered alcoholic iodine solution" and watched the individuals fall in the solution to determine which end, anterior or posterior, was directed downward. More than half of them, according to him, sank without any definite orientation of the axis or in "beliebiger Querstellung," while a greater part of the rest sank with their posterior ends directed downward, and the other part with their anterior end directed downward.

From these results and others, he concluded that "the orientation of the *Paramecium* with the long axis vertical and in the direction of gravity would not be brought about by a purely physical effect" (11, p. 455). Jensen also tried similar experiments with *Euglena viridis*, and found that the majority of them sink with their anterior end downward (11, p. 457).

Recently Lyon rightly criticized Jensen's experiments in killing the animals in the alcoholic iodine solution, saying that "the centers of buoyancy and gravity might have been changed through distortion or through localized changes of density in the protoplasm" (17, p. 423). Lyon having questioned Jensen's method, "made use of the fact that the animals when centrifuged

strongly are passively thrown to the bottom of the tube." "A glass tube was drawn into a capillary so fine that paramecia could not turn around in it. The capillary end was dipped into distilled water and the tube filled by capillarity . . . ; then the end was sealed and a drop of water containing paramecia was introduced into the large part of the tube. The tube was fastened to the centrifuge so that the capillary pointed away from the axis of the machine. After strong rotation the capillary was examined under the microscope. The organisms were invariably found with anterior ends directed towards the closed end of the tube." From these results, Lyon concludes: "It is therefore certain that passive paramecia would fall head end down, and that their negative geotropism is the result of an active process on the part of the animals. Jensen's view in this respect is fully confirmed and the mechanical theory is to be laid entirely aside" (17, p. 423).

Judging from Lyon's results, the anterior end seems to be "heavier" in spite of the larger shape of the posterior end. Thus Verworn's theory as well as Davenport's supposition of a static equilibrium (2, p. 122) are found untenable.

Very recently, however, Harper has taken up the problem and in his two articles has again revived the "mechanical theory" (3–4). He adopted the method of Kreidl (13) and that of Prentiss (21) who made crustaceans take in iron-filings as "statoliths" and then applied a magnet. Harper made *Paramecium caudatum* ingest finely divided iron prepared by the alcohol method. In so doing he varied the time for the ingestion of iron, from 30–40 seconds to five minutes. He seems to have obtained his best results when the ingestion of iron was allowed to continue for one minute. "The treated animals," he says in his first article, "swarmed to the top *more quickly* and formed a denser ring there than in the control" (p. 996). From this result and others, Harper drew the conclusion that "the pull of gravity on the *heavier* posterior end may produce a tipping effect which is able to *orient passively but is too weak to stimulate.*"[1] And he believes that here "we have in the normal, quiet, geotropic reactions of *Paramecium* an example of a *purely mechanical* tropism"[1] (3, p. 998).

[1] All italics not in the original.

Harper also claims that the posterior end of normal paramecia is heavier, because they assume the same position in respect to gravity as do those paramecia which have ingested iron in their posterior ends, and which are therefore undoubtedly heavier. But a generalization from such an experiment is not always safe. Spiders, for instance, whose posterior region or abdomen is unmistakably larger and heavier than the anterior region, always orient the head downward *at rest*.[1] Through his experiments, therefore, Harper is hardly justified in drawing his conclusion. Again, according to him, there is no negative geotropism of an active kind on the part of the normal paramecia, though Lyon thinks there is, because the "pull of gravity" "is too weak to stimulate," unless it is augmented by "a greater force" like a strong centrifugal force. "When, however, a stronger force is substituted for gravity so as to produce a sudden orientation, the irritability is affected, and the animal reacts to the change" (3, p. 999).

This conclusion was based on the rheotropism of paramecia. "When . . . the centrifugal effect," he says, "exceeds the pull of gravity and produces too sudden an orientating tendency, this may act as in the ordinary rheotropic reaction against a current. When strongly centrifuged the animal takes a position so that it moves in the water just as the water moves past it in the rheotropic response." "If it allowed itself to be oriented by the centrifugal force with posterior end in advance its relation to the water would be the reverse of what it is in the rheotropic response" (3, p. 998).

From this point of view Harper criticizes Lyon's results. According to the former, "the inference that the anterior end is heavier is contrary to what the shape of the body would indicate, unless the heavier particles are located anteriorly." So he suggests "as an explanation of Lyon's experiment that in strong centrifugalization the same effect is produced at the outset as by mechanical agitation, *i. e.*, the reaction changes to positive." After a repetition of Lyon's centrifugalization experiments,

[1] Moreover, the larvæ of a marine annelid, *Arenicola*, the anterior end of which is larger and heavier than the posterior end, are negatively geotropic. The writer has found by centrifuging that its anterior end is heavier than the posterior end, and further experiments are still in progress (1913).

"the explanation here given" "seemed to him the most satisfactory explanation of the fact that all are found to move with the anterior end outward" (3, p. 998). This suggestion of Harper is worth serious consideration as a criticism of Lyon's experiments, and must not be overlooked; because we know from Sosnowski (23, p. 133) and Moore (19, p. 243) that paramecia which have gathered at the top of a tube often become positively geotropic by a "shock" or "mechanical agitation." The present writer also found this to be true. But two series of questions arise: (*a*) What makes the animals swim downward after the mechanical shock or agitation? If the "pull of gravity" "is too weak to stimulate," it should not produce a positive geotropism any more than a negative one, because the pull of gravity always remains the same before and after the mechanical shock. In Lyon's centrifugalization experiments, however, the "substituted" centrifugal force continued to act until the animals were carried into the capillary tube where they could not turn around. Centrifugalization and temporary mechanical agitation have thus different effects. And (*b*), if the posterior end of the animals is "heavier" than the anterior end, as Harper claims, is it possible that the animals, when *strongly* centrifuged, are really "able to react at the outset of centrifugalization," so as to direct themselves with the "lighter" anterior end away from the axis of the centrifuge? If we believe in a mechanical explanation in this case, as Harper does in the normal case of the "passive orientation," we should hardly expect that this would be the case, because Lyon employed too strong a centrifugal force and the mechanical effect is proportioned to the force acting. It seems to us therefore that the question remains unsettled, though Harper seems satisfied with the mechanical theory.

In his second series of experiments, Harper used a strong electromagnet applied to the iron-ingested paramecia. From the results, he concludes that "the passive sinking of the posterior end is able to orient the animal into a position of gravity equilibrium with the anterior end up" (4, p. 189). As to the magnetic effect, he thinks that it caused in the iron-ingested animals "an upward streaming in the stronger part of the field. Those heavily

loaded animals which move upward in this stream after dispersing above into the weaker part of the magnetic field tend to sink again and cause a return circulation to the bottom. The magnet is effective in producing this circulation by diminishing the effect of gravity on animals containing iron. It also exerts a passive pull upon them and they gradually swing into their finally oriented position in a vertical path under the combined influence of the magnet and gravity" (4, p. 189). Harper could not get any definite results showing a direct effect of the magnet. "The movement toward the magnet," he says, "is the most diffused feature of the circulation, so that superficially it might be set down as the result of the random movements of the animals" (4, p. 185).

1. *Experiments on Paramecium caudatum.*

Experiment a.—Judging from the results of both his experiments, Harper contributes little to the solution of our problem, except his criticism on Lyon's experiments already mentioned. Hence the present writer looked for a method that would obviate Harper's objection to Lyon's conclusions as much as possible. He took advantage of the fact that the paramecia are positively rheotropic, that is, they show the positive reaction to water currents as already stated. For this purpose, he repeated Lyon's method of centrifugalization with a capillary tube like that described by Lyon. A glass tube about 4 mm. in diameter and 7 cm. in length was drawn at the middle into a capillary tube so fine that paramecia (and spirostoma) could not turn around in it.

The parts of *abc* and *cde* were both about 3.5 cm. long. The end of *e* was "sealed" in Lyon's case, but it was *left open* in those used by the writer for the following reasons. The heavier ends of the animals, when centrifuged in the tubes so prepared, are not only passively thrown to the end of the tube away from the axis of the centrifuge so that they close the open end of the tube themselves, but at the same time the current of water is also thrown in the same direction with the animals. The animals being positively rheotropic, as Jennings found (9, pp. 468–473),[1]

[1] The present writer also found that their positive rheotropism is often stronger than their negative geotropism.

they tend naturally to orient themselves in the tube with their anterior end toward the axis of the centrifuge as far as their rheotropism is concerned; thus against the current. Therefore, to some extent, this positive rheotropism, when the animal is centrifuged, will tend to prevent the downward, *i. e.*, positive "orientation" of *Paramecium* to gravity "at the outset of the centrifugalization" as by mechanical agitation. In such a case, if the posterior ends of the animals are "heavier" than their

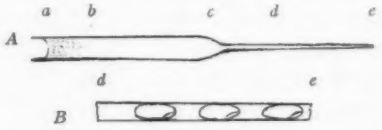

FIG. 1. Tube *A* ready for centrifuge, and *B* a part of capillary after centrifuging, magnified, showing paramecia forced down head first (a slightly modified Lyon's preparation).

anterior ends, as is supposed by Harper, it would be expected that their posterior ends would be passively thrown to the end of the tube *away from* the axis of the centrifuge while the anterior ends would be "oriented" against the water current *toward* the axis. Thus this method obviates in a measure Harper's criticism rendered on Lyon's experiment. Now the writer put some distilled water in the part *bc* of the tube (see diagram), say about 2.5 cm. long, and then added by a small pointed pipette two or three drops of a dense suspension of paramecia, absolutely free from sediment, in the part *ab*. The tube was fastened to the centrifuge as soon as possible, so that the capillary end when centrifuged should be pointed away from the axis of the machine. After centrifuging at a rate of about fifteen revolutions per second for 10 seconds, the capillary was examined under the low power of the microscope. Every animal was without exception found with its anterior end directed toward the open, outer end of the capillary tube in every trial. This was carefully repeated, varying the speed of the centrifuge, that is, with both higher and lower speeds than 15 revolutions per second. The amount of distilled water in the part *bc* was also varied from a column 2.5 cm. long to zero. But this made no difference.

The writer calculates the centrifugal force which he used for the above experiments as follows: The revolutions with the radius about 4 cm. long being about 15 per second, the centrifugal force is given by the formula

$$f = M \cdot \frac{(2\pi r \cdot n)^2}{r},$$

where M = mass in grams, r = radius of circle, n = number of revolutions per second. This gives for $r = 4$ cm. and $n = 15$, $f = M \cdot 35{,}494$ dynes or $M \cdot 36.2$ grams. Moreover, the revolutions were increased to about 20 per second, more or less, as already stated, and then the centrifugal force was about $M \cdot 59{,}101$ dynes or $M \cdot 60.32$ grams. Under these conditions, is it thinkable that such a small animal as *Paramecium* "is able to react at the outset of centrifugalization" against the centrifugal force of the above calculation so as to direct itself with its "heavier" posterior end toward the axis of the centrifuge as Harper claims?

Moreover, Harper states that "an excessive amount of iron may *overload*[1] the animals apparently and caused them to aggregate at the bottom" (3, p. 995). Even though an "overload of iron" might be *quite excessive* for the animal, it could not possibly be comparable with the centrifugal force which the writer used. Nevertheless, Harper thinks that in the case of the *strong* centrifugalization "the animal is able to react" with the supposedly lighter anterior end directed away from the axis of the centrifuge. Such mechanics we can hardly understand.

Experiment b.—With the same point in mind, the writer made another series of experiments with iron-ingested paramecia, as suggested by Harper's experiments. He mixed about a half-gram of finely divided iron prepared by the hydrogen method with about 2 c.c. of a dense culture of paramecia free from sediment. The iron was kept in suspension by drawing the water in and out with a pipette for about 5 minutes. All the paramecia so treated ingested iron in the posterior end of the body. Two or three drops of the culture containing paramecia

[1] Italic not in the original.

so prepared were put in a tube as described in the preceding experiments; the tube was already filled between the points *bc* with about 0.2 c.c. of distilled water. The tube then was centrifuged. The procedure was exactly the same as the preceding experiment. In this case, every animal was found with its posterior end unmistakably directed toward the open outer end of the capillary tube. From the presence of the ingested iron in the posterior end of the animals, we should expect this end to be *heavier* than the anterior end, as Harper says. It is no wonder then that the "heavier posterior end" in this case was passively thrown away from the axis of the centrifuge, as the heavier anterior end was in the normal case. This is the only possible conclusion we can draw. Lyon's view in this respect is fully confirmed, and Harper's explanation is not satisfactory.

Experiment c.—The writer repeated Harper's experiments on the paramecia which had ingested iron as already described. He placed one control tube containing a thick culture of normal paramecia, side by side with a series of five tubes, *a* to *e*, containing iron-ingested animals which were exposed to the iron-containing medium for different periods, as follows: *a* for 30–40 seconds, *b* for two minutes, *c* for 3.30 minutes, *d* for 5 minutes, and *e* for 10 minutes. All of the tubes were in a vertical position. About 18 minutes later, in the control tube, about 70 per cent. of the animals had gathered at the top, 20 per cent. at the bottom, and the rest were scattered through the tube. In all the treated tubes, about 80 per cent. of the animals had gathered at the top and the rest were scattered through the tubes; except in tubes *a* and *b* where there was on the bottom of each tube a small dense gathering which disappeared about 10 minutes later. After about two hours and fifty minutes, in all treated tubes, almost all the animals had gathered at the top, especially in tube *e*, and a very few were scattered here and there, while in the control, 70 per cent. were still at the top and the rest at the bottom.

According to Harper, the ingested iron "particles are at first lodged in the posterior end," and "the changed response is coincident with this condition and tends to disappear as the particles become more evenly distributed through the endo-

plasm" (4, p. 181). The writer continued his observations for about three hours and found that no such change in reaction occurred. At the end of the time, almost all the animals in his case were at the top of the tubes.

About 16 hours later, in the treated tubes, all the paramecia were dead except a few in tubes *a* and *b*. They may have died earlier than this, as the writer did not have a chance to observe them in the meantime. He examined the dead animals under a microscope, but the iron particles were not noticeably "distributed through the endoplasm"; some animals were found vacuolated and many must have broken into pieces, because few dead animals were found. On comparing the above observations with Harper's, we find some differences. According to Harper, "the paramecia rid themselves of the iron after the course of a few hours without apparently harmful effects from a small amount" (3, p. 995). It may be premature to assume a chemical effect on the protoplasm of the iron-ingested paramecia, but we may expect some abnormal reactions of the animals, besides the purely physical; because we saw that all the paramecia treated for 3.5, 5, and 10 minutes died within at least 16 hours, as already stated. In other words, the more iron the animals ingested the quicker their death resulted.

From the above results it is clear that the iron-ingested paramecia are more apt to be negatively geotropic than the normal ones, as Harper has shown. Since the iron is ingested in the posterior region, which presumably is thus made heavier than the anterior region, Harper's view of a "passive orientation" may be true in this particular case. The writer does not mean, however, by "passive orientation," as Harper does, that the "pull of gravity" "is too weak to stimulate" in any case. In point of fact, the normal *Paramecium* orients its anterior end upward not because that end is heavier but *in spite* of it. Of course, therefore, when the posterior end is rendered heavy by iron the orientation is easier. It is here that the "mechanical theory" deserves some credit. It gives us one of the factors of orientation of some animals—like iron-ingested paramecia—against the force of gravity. The "stern-heaviness" or "bow-heaviness," therefore, may be one of the conditions of stimulation

which bring about orientation. But it is not the only condition because, as we have already observed, the negative geotropism of the normal paramecia may be altered by a mechanical agitation to a positive geotropism. We must, therefore, hold with Moore (19, pp. 243–244), Jennings (10, p. 99), and Lyon (17) that there are some additional internal or physiological conditions of orientation in such a case.

Experiment d.—In the experiments on iron-ingested paramecia the writer used an electromagnet not as strong as that used by Harper, employing a binocular microscope placed horizontally for the observations of the movements of the animals, so adjusted that the experimental tube was readily observed. The writer found in these experiments that many animals swam upward, while others swam downward, and a few others toward and away from the electromagnet, which was applied at one side of the tube. According to Harper, the path of paramecia so treated represents the resultant of the pulls of gravity and of the electromagnet. The writer saw some behave in this way, but he could hardly specify it as characteristic. He was unable to detect any orientation with respect to the magnetic force. Possibly the weak electromagnetic force in the writer's case might be the cause of the difference between his results and those of Harper. But even with such a weak force it was observed in many cases that the pull of gravity was easily obscured by the pull of the electromagnet. In a few cases it was observed that the individual when passing in front of the magnet was passively drawn backward toward it when the electric current was connected and moved in a straight line away from it when the current was disconnected. Such cases indicate that the pull of gravity has no effect on the animal in comparison with that of the electromagnet.

2. *Experiments on Spirostomum teres.*

Experiment a.—Spirostomum teres is one of the largest of unicellular organisms. The average length of those on which the following experiments were made was about two tenths of a millimeter. Spirostoma from a wild culture were centrifuged to determine their position in a capillary tube in the same way

as in the preceding experiments. The majority of the animals were found with anterior ends directed toward the open outer end of the capillary tube. Owing to the animal's extreme contractility and long form, the writer could not obtain such invariable uniformity of results as he did in the case of *Paramecium*. Nevertheless, the proportion thus directing their anterior end with reference to the centrifugal force was sufficiently great to show that the position was not accidental.

Experiment b.—An attempt was made to repeat the iron-ingestion experiments on spirostoma, but without success. The writer mixed iron filings with about 2 c.c. of the "wild" culture containing a dense suspension of spirostoma in the way that is already described. Within five minutes, all of them broke into pieces beginning at the anterior end and continuing to the posterior, even though none of them ingested iron. Here we see the great injury of iron to the animals; so that the iron ingested by paramecia might not be "harmless" to these animals or at least it might produce some chemical effect on them.

III. The Pressure Theory and its Criticism.

Jensen, having been dissatisfied by Verworn's mechanical theory, proposed the "pressure theory" of geotropism (11, pp. 462–464). According to Jensen, the geotropic orientation of paramecia is determined by the difference in hydrostatic pressure between the upper and lower surfaces of the animal. In the negative geotropism, therefore, the animal moves from the place of high pressure to that of low; in positive geotropism, the animal moves from the place of low pressure to that of high (11, pp. 462–463). Davenport first criticized this theory of Jensen's, and suggested a third theory (2, pp. 122–123). Jennings (9, pp. 473–477) and Lyon (16, pp. xv–xvi) independently disproved Jensen's theory from a physical point of view.

Jennings calculated that "the difference in pressure between the two sides of the organisms is only 1/1,000,000 of the pressure acting everywhere on the surface. Furthermore, Jensen showed that the reaction still occurs when the atmospheric pressure is more than doubled; the effective difference in pressure would be less than 1/2,000,000 of the general pressure" (9, pp. 475–

476). Lyon's calculation was practically the same as Jennings, *i. e.*, 1/1,040,000 (16, p. xv). Since it is well known that the "threshold differential required for the perception of differences in pressure" in man is "about 1/10," both investigators cannot believe that a differential of 1/2,000,000 or 1/1,040,000 is perceptible by paramecia. With regard to the difference in pressure between the anterior and the posterior ends, Jennings thinks that the posterior end being only slightly sensitive to a touch to which the "anterior end reacts violently," "an increase of pressure on the posterior end" "would cause no reaction whatever" (17, p. 476). Neither Jennings nor Lyon calculated the difference in pressure between the anterior and posterior ends of the organism, but this is easily made as follows: The atmospheric pressure being 10,000 mm. of water, and the average length of the paramecia which the writer experimented on about 0.2 mm., the writer calculates the difference in pressure between the anterior and posterior ends of the organisms to be about 1/50,000 of one atmosphere. If "the reaction still occurs when the atmospheric pressure is more than doubled," as Jensen showed, "the effective difference in pressure would then be" about 1/100,000 of one atmosphere. It is hardly conceivable that the difference of either 1/50,000 or 1/100,000 of one atmosphere is perceptible to paramecia. Moreover, Lyon's experiments have shown that "geotropism is intensified in a centrifuge tube which is open away from the axis and in which, therefore, no increase of pressure above atmospheric is possible" (17, p. 431). On the basis of such various evidence, we ought to abandon the pressure theory of geotropism in *Paramecium*.

IV. The Resistance Theory.

A third theory of geotropism, proposed by Davenport, is based on the assumption that the animal "experiences greater resistance (friction + weight) in going upwards even to the slightest extent than in going downward (friction − weight)" (2, p. 122). Lyon calls this the "resistance or weight theory" (17, p. 426). This theory of Davenport was once accepted by Jennings (9, pp. 477–480), though now he rather favors Lyon's theory (10, p. 77) which we shall consider later.

If the weight of organisms or their resistance to the pull of gravity is the only cause of geotropism, negatively geotropic animals should become positively geotropic, and positively geotropic animals should become negatively geotropic in solutions of greater specific gravity than their own, as Platt points out (20, p. 31). Platt at the suggestion of Davenport attempted in vain to solve this problem, because she could not find "paramecia which showed decided geotropic reaction" (20, p. 32). The first approach to this problem was to determine the specific gravity of the *Paramecium*. Jensen first reported it as 1.25 (12, p. 544). He killed *Paramecium aurelia* in solutions of potassium carbonate (12, p. 543) and determined their specific gravity when they were suspended in a solution of known specific gravity. But as Platt has shown (20, p. 34), the specific gravity of the animal when so treated was certainly increased in the solution to some extent. The value of the specific gravity of paramecia obtained by Jensen, therefore, is hardly worth serious consideration. Platt then tried to determine the specific gravity by killing the animals in water to which was "added a few drops of 0.5 per cent. acetic acid." She "also killed paramecia by the fumes of osmic acid." Then she placed the killed paramecia "in a gum-arabic solution of 1.018 specific gravity," whereupon they "remained long suspended in the solution" (20, pp. 35–36). So she thinks that the specific gravity of paramecia so treated "is about 1.017" (20, p. 38). But Platt's method was no better than Jensen's. As she observed that the "reagents caused the paramecia to change their shape somewhat in dying, by becoming wide and shorter," we are not justified in assuming that the specific gravity of the dead animals remained the same. "On the death of the cell," according to Lillie, "there follows a marked and permanent increase in the general permeability, and this change is always associated with a permanent fall in the potential difference between surface and interior" (14, p. 196). Furthermore, as Lyon rightly points out, a viscous gum-arabic solution is likely to hold suspended for a long time even bodies of considerably different density "unless a greater force than gravity be used to separate them from the solution" (17, p. 427). We can therefore place little value on either Jensen's or Platt's results.

Lyon then adopted a method "by which the density of small organisms could be found without any injury or change in them." He "made use of the hæmatocrit attachment of the centrifuge by which very high velocities" (from 10,000 revolutions to 12,000 per minute) "of rotation and consequent high centrifugal force are secured. At the bottom of a *small*[1] tube was placed *a little*[1] gum-arabic solution of known density; above this a *few*[1] drops of water containing paramecia. The tube was then centrifuged about one minute." He thus found that "an average density of paramecia is about 1,048 or 1,049" (17, p. 427).

1. *Experiments on Paramecium caudatum.*

In the results of Jensen, Platt, and Lyon, however, the difference of density is so great that the present writer thought it well to repeat Dr. Lyon's experiments, under his direction, with some modifications. The writer prepared a gum-arabic solution using distilled water. The acid of the solution was carefully neutralized with sodium carbonate. But he regrets that he forgot to dialyze it. He made a series of 40 solutions of different densities from 1.02 to 1.05 in Naples jars. The specific gravity of each was carefully determined by means of a finely graded hydrometer. After some trials the solutions above and below the densities given in the following table were found unnecessary. Then he prepared many small tubes about 12.5 cm. long and 0.4 cm. in diameter, one end of which was sealed. In each trial, two of those tubes were filled about 11 cm. high with gum-arabic solutions of known specific gravity, *e. g.*, the one 1.02 and the other 1.021. This pair was centrifuged to drive the air out. Then on the top of each solution in the two tubes, *one* drop of the culture containing dense paramecia was added by a very small pointed pipette. The tubes so prepared were again centrifuged for one minute with a speed of about 1,300 revolutions per minute. The distribution of the animals in both tubes was then carefully compared. All 40 different solutions having been thus tested, the nearest possible density of the animals was obtained:

[1] Italics not in the original.

I. Tube 1. Density of gum-ar. sol. 1.034, 1,300 revolutions; a few go to bottom.
Tube 2. Density of gum-ar. sol. 1.035, 1,300 revolutions; very few go to bottom.

II. Tube 1. Density of gum-ar. sol. 1.036, 1,300 revolutions; 2 or 3 go to bottom.
Tube 2. Density of gum-ar. sol. 1.037, 1,300 revolutions; none go to bottom.

III. Tube 1. Density of gum-ar. sol. 1.038, 1,300 revolutions; a few go to lower part than middle.
Tube 2. Density of gum-ar. sol. 1.039, 1,300 revolutions; some go to middle.

IV. Tube 1. Density of gum-ar. sol. 1.030, 1,300 revolutions; many go to bottom.
Tube 2. Density of gum-ar. sol. 1.043, 1,300 revolutions; many stay at top.

The writer found thus that the density of the *Paramecium caudatum* is about 1.036 or 1.037. To make sure of the results, three different series of the same experiments were made with different cultures in each series. From two cultures, practically the same results were obtained as the above, but it was found in one culture that the animals when centrifuged were so weak that they were all broken into pieces on the bottom.

In comparison with Lyon's 1.048 or 1.049, we find a difference of about 0.012. Although the difference does not seem very great, it is great for such small organisms. Accordingly at the suggestion of Dr. Knowlton and Dr. Lyon, the writer tested the hydrometer which he used for the measurement of the density of the gum-arabic solutions, by means of a density bottle with Dr. Lyon's help. It was found that the weighing difference between the hydrometer and the bottle was about 0.003. If the test was correct, we may add this difference, 0.003, to 1.036 or 1.037, which makes the density 1.039 or 1.040. If so, the difference between Lyon's 1.048 or 1.049 and the writer's 1.039 or 1.04 becomes a little less.

The only possible source of error in Lyon's experiments is that he may have placed too much "water containing paramecia" above "*a little* gum-arabic solution" in the "*small*" and short tube which he adopted for the hæmatocrit attachment. Under such circumstances, possibly the animals when centrifuged carried some water with them into the gum solution

and lowered the known density of the latter. Nevertheless, there may exist some difference in the specific gravity of the animals in different cultures, as seems to be indicated by the experiments described. This must be borne in mind.

Then the writer prepared a series of 10 tubes about 12.5 cm. long and 0.4 cm. in diameter, in each of which was a definite gum-arabic solution of known density—from 1.034 to 1.044—thoroughly mixed with about two drops or slightly less of the culture containing dense paramecia, filling each up to 12 cm. Three drops of paraffine oil were placed on top of the solution "to exclude oxygen and a possible consequent chemotropic gathering." The writer's observations upon the paramecia so treated agreed with Lyon's in all respects. His words may, therefore, be quoted: "Invariably in all solutions, whether of equal, greater, or less density than the animals, the latter slowly rose and formed a dense ring near the top. The response was slow, as the velocity of swimming in such viscous solutions is extremely small. Two or three hours, or over night, was often necessary. But often the rising ring of animals could be seen in twenty minutes or less. It was typical geotropism very much slowed" (17, p. 428).

The animals so treated lived 5 days in all the solutions above mentioned, though the number of the animals had noticeably decreased at that time. A week after, all the animals in the solutions of densities 1.044 to 1.037 had died, though a few animals in the solutions from 1.036 to 1.034 lived for 13 days. It is rather interesting therefore to note that the animals in the solutions of the greater densities than their own died earlier than the animals in the solutions of the lesser densities. This may not be simply due to density, but density may be at least one of the causes of death of the animals.

To avoid the criticism that the specific gravity of the animals may have been increased by a prolonged stay in such solutions, the writer carefully tested the matter, as Lyon did. After several tests, he found that the animals had increased their density on an average between 0.002 and 0.003 after 13 hours in the solutions. This was determined by centrifuging tubes in which the animals were suspended in solutions of known densities.

The distribution shown by the animals was then compared with the distribution shown in the original determinations of their specific gravity. If the animals in the solution of 1.037 increased their density about 0.003 after 13 hours, they should be distributed in the tube when centrifuged at this time as they had formerly been in the solution of 1.034. The results of Lyon and those of the writer agree fairly with one another in this respect (17, p. 428).

From the results of all the above experiments, we have found that the weight or resistance theory of geotropism is untenable. We have found that no animals which are negatively geotropic become positively geotropic in gum-arabic solutions of greater specific gravity than their own. Thus Lyon's results in all respects are fully confirmed except as regards the exact value of the specific gravity of the animals.

2. *Experiments on Spirostomum teres.*

The same experiments as the above were made with Spirostoma in the same ways. The density of the animals was found:

I.	Tube 1.	Density of gum-ar. sol. 1.022, 1,000 revolutions; some go to bottom.
	Tube 2.	Density of gum-ar. sol. 1.023, 1,000 revolutions; a few go to bottom.
II.	Tube 1.	Density of gum-ar. sol. 1.024, 1,000 revolutions; 4 go to bottom.
	Tube 2.	Density of gum-ar. sol. 1.025, 1,000 revolutions; none go to bottom.
III.	Tube 1.	Density of gum-ar. sol. 1.026, 1,000 revolutions; a few go to lower part.
	Tube 2.	Density of gum-ar. sol. 1.027, 1,000 revolutions; a few go to middle.
IV.	Tube 1.	Density of gum-ar. sol. 1.019, 1,000 revolutions; most go to bottom.
	Tube 2.	Density of gum-ar. sol. 1.030, 1,000 revolutions; most stay near top.

These experiments show that the specific gravity of *Spirostomum teres* lies between 1.024 and 1.025, or very near to 1.025. If we add 0.003 to the 1.025, as we did before, it becomes about 1.028.

Platt obtained a specific gravity of spirostoma of about 1.017 (20, p. 34), which varies by about 0.008 from 1.025. As we have

already pointed out, Platt's method was not as accurate for obtaining the density of the animals as Lyon's. We may therefore consider our results to be the more accurate.

Then nine tubes about 12.5 cm. long and 0.4 cm. in diameter were prepared. They were filled up to about 12 cm. with the solutions of known density—from 1.24 to 1.032—thoroughly mixed with about two drops of the "wild culture" containing dense spirostoma, free from sediment. Three drops of paraffine oil were placed above the solution in each tube.

The negative geotropism of spirostoma is not so marked as usually supposed, and their movements under these conditions were much slower than those of the paramecia in the preceding experiments. About two hours after placing in the solutions of the same density as their own a few rose up to about the middle part of the tubes; a still larger proportion so rose in the solutions of greater densities than their own, but in the solution of 1.024 all were on the bottom, while in that of 1.025 a few rose from the bottom of the tube to the height of 2.5 cm. This suggests that the buoyancy in the solutions of greater densities than their own helped the animals to rise. But about 13 hours later, nearly all the animals in the solutions rose up to near the top. Two weeks later some spirostoma were living in all the tubes except one of 1.027 which was broken 4 days after treatment. At that time the writer had to leave Woods Hole. Meanwhile the number of the animals considerably decreased, but in some tubes a few individuals divided.

V. The Statocyst Theory and Its Criticism.

A fourth theory of geotropism proposed by Lyon is based on the assumption that the *Paramecium* contains protoplasmic materials of different specific gravity. "For internal stimulation the relation of the parts of the cell to each other must be changed in some way by grvaity. Stresses or pulls which occur when the organism is in one position with respect to the vertical, must be changed in another position" (17, p. 429). This is called the "statocyst theory" of geotropism in the *Paramecium*. It is worth while to mention, with reference to the general theory of geotropism in animals, that Loeb in 1897 (15, pp. 446–449) had already suggested a similar theory.

Lyon furnishes evidence (though not conclusive) for his theory as follows: "The animals were strongly centrifuged for several minutes in the hæmatocrit attachment. Microscopic examination showed that certain dark granules originally distributed were now aggregated in one end, usually the anterior. It is thus seen that differences in specific gravity exist in the protoplasm of this animal" (17, p. 430).

The writer tried, by means of the centrifuge, to test Lyon's results, but must confess that he could obtain nothing definite. But he thinks now that he ought to have adopted some chemical methods (stains) which might possibly show better results. But since all theories based on the results of external stimulation have been shown above to be fallacious, this theory of Lyon's seems from a physico-chemical point of view to be the most possible and the most reasonable. If this theory is tenable, the matter of the heaviness of either anterior or posterior end is of very little significance in such a unicellular organism as *Paramecium*, because even the lighter end of the organism may possibly contain protoplasmic materials of a specific gravity greater than that of this region taken as a whole.

VI. Conclusions.

So far as the results of our experiments and analyses are concerned, the geotropism of paramecia is due chiefly, if not entirely, to the internal conditions of the organisms rather than to external ones. The results of our experiments have shown that the anterior ends of the animals are heavier than their posterior ends. This fact gives no direct clue to our problem, but furnishes strong evidence against the mechanical, that is, external, theory. On the other hand, if we suppose with Harper that the posterior ends of the animals are "heavier" than their anterior ends, that the "pull of gravity" "is too weak to stimulate the organisms," and that the consequent negative geotropism is due to the orientation of the animals in a "purely mechanical" way, we have no explanation for the lack of uniformity in response to the same stimulus under varying conditions. For instance, when given a "shock," the normal paramecia which form a ring at the top of a tube become positively geotropic and swim

downward, even though the "shock" lasts only a moment. Moreover, as Sosnowski (23, p. 134) and Moore (19, pp. 239–241) have shown, the animals are negatively geotropic at 2° C., while they are positively geotropic at 30° C. As we have already pointed out, certain spiders whose posterior region is much heavier than the anterior, nevertheless orient themselves, when at rest with their head down. Therefore we conclude that without consideration of the internal factors, we have difficulty in explaining the facts, even though we do not absolutely reject the external factors.

A possible method which the writer intends to try later would consist in the following: Immediately after centrifuging a capillary tube containing paramecia, and in which the latter cannot turn around, we may stain them with some dyes and determine the effects of the centrifugal force upon the protoplasmic materials of the organisms. The application of this or similar methods is of the utmost importance for the problem of geotropism not only in paramecia but in animals in general.

VII. Summary.

1. *Paramecium caudatum* and *Spirostomum teres* assume a position with their anterior ends directed away from the axis of the centrifuge. Their anterior ends, therefore, must be heavier than their posterior ends. If so, the negatively geotropic orientation is an active process and the mechanical theory cannot be held.

2. It is not conceivable that the animal could be sensitive to slight differences in pressure such as we have computed must exist between the two sides or the two ends of its body. Therefore the pressure theory is not tenable.

3. *Paramecium* and *Spirostomum* in the gum-arabic solutions of greater, equal, and less specific gravity than their own are still negatively geotropic. Therefore the resistance or weight theory is not correct.

4. The specific gravity of living *Paramecium caudatum* is about 1.037 or 1.037 ± 0.003.

5. The specific gravity of living *Spirostomum teres* is about 1.025 or 1.025 ± 0.003.

6. The statocyst theory of geotropism is the most tenable.

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PURE LINE INHERITANCE AND PARTHENOGENESIS.

H. E. EWING.

INTRODUCTION.

Ever since Johanssen, by his epoch-making researches with self-fertilized strains of beans, established his pure line conception of heredity, other investigators have been extending this conception so as to have it include many forms which do not reproduce sexually. In fact some of these investigators hold that all selections made within isolated strains of asexually reproducing plants and animals will fail to alter in the least the fraternal mean of succeeding generations.

Some careful work already has been done with lower organisms, which shows that Johanssen's pure line conception holds true for forms reproducing by fission or budding. Up to the present time no extensive work has been done with parthenogenetic animals. In fact the only paper which I have found dealing with selections among parthenogenetic forms is one by Kelly. Kelly worked with an aphid, *Aphis rumicis*, but did not carry his selections beyond two generations. However, he came to the conclusion that, "the progeny does not inherit the somatic idiosyncrasies of the parent, but does inherit from the underlying germ plasm common to all."

During the winter of 1910–'11, I began a series of experiments along pure lines with the cabbage aphis, *Aphis brassicæ* Linn., but while moving about from one part of the country to another I confined the individuals too closely in their breeding cells with the unfortunate result that they all died.

Again during the summer of 1912 I took up similar experiments with our common black cherry aphis, *Myzus cerasi* Fab., but before I obtained a sufficient number of generations to make the results reliable, cold weather set in causing the sexual forms to appear, thus spoiling my results.

While working this last winter with the European grain aphis, *Aphis avenæ* Fab., I noticed its great variations, and since the

species was already reproducing parthenogenetically under laboratory conditions, I decided to take up for the third time heredity work with a parthenogenetically reproducing insect. This time my efforts have been rewarded with some measure of success. Most of the results obtained up to the present are here submitted.

Life History and Habits of Aphis avenæ Fab.

Before submitting the results of these experiments, and entering upon a discussion of them, I shall review briefly the life history and habits of the species under consideration.

As is true of aphids in general, this species propagates itself chiefly by means of parthenogenesis, but under certain conditions (probably due chiefly to climatic changes) the true sexes appear, mating occurs, eggs are deposited, and the parents die. In the case of this species, according to Pergande, the true sexes do not appear annually under out-of-doors conditions; but the asexual females live over the first winter, the true sexes being produced the next fall. Thus two years are required to complete the cycle from egg stage to egg stage.

Aphis avenæ lives on a large number of plants, but is especially injurious to small grains and grasses. It will frequently winter over on apple, pear, plum, or other deciduous trees. The eggs that have passed the winter out of doors usually hatch about the middle of March in most of the eastern states. It takes these young aphids about a month to reach maturity; when they are called the stem-mothers. The offspring of these stem-mothers are all asexual females, and produce parthenogenetically, for several or many generations, other asexual individuals. These asexual females are of two classes; winged and wingless. Although the winged or wingless condition of these two classes constitute the chief ground for their differentiation there are other differences also, but generally these are not very important.

During the warmer part of the season the asexual females reproduce very rapidly, and the time required for the complete life cycle may not be more than six days. During the colder parts of the year two weeks or even more may be required for the completion of the life cycle. The asexual individuals vary

greatly in their period of longevity and in their fecundity. Some will die in a few days after reaching maturity, and will leave few or no offspring. Others may live for two weeks or more, and leave at least a score of offspring.

The young when born are quite similar to the adults except in size. They at once begin to feed, and after molting three or four times they reach maturity. Although the immature individuals are quite content to settle on the same stalk next to their mother, and to remain there throughout their period of growth, when they reach maturity, especially if they are winged, they are restless, and generally leave the plant upon which they have been reared.

VARIATIONS.

This aphis is quite variable in several respects. Pergande, who has made a special study of the species, states: "I have found a certain range of variation in the comparative length in the joints of the antennæ, as well as in the nectaries of the progeny of the apple louse [*Aphis avenæ*], the extreme forms of which may easily induce superficial students to consider them as distinct. Large series, however, of the various forms, more or less due to the season or abundance of food, have convinced me that all of them belong to but one species."

I cannot agree with Pergande in attributing these variations entirely to the season or the food supply. For ten generations I have reared these aphids exclusively on young wheat shoots, and I find that most of these variations obtain even in their extremes when all individuals are reared on the same diet. However, the food supply certainly does affect some of the variations, but most of them originate without a change in the amount or nature of food taken. What is more significant, I find these variations existing among offspring of a single female reared under identical conditions and even find the antenna on one side differing from the antenna on the other side in the same individual. In regard to this latter point, however, I find that for a large majority of the individuals examined the antennæ on the two sides of the same individual are the same or nearly so.

After studying the different characters which were available I finally decided to use those of the antennæ. The variations in the relative lengths of the third and fourth segments were observed and employed. It is interesting to note that these characters are the same as those I selected two years ago when working with *Aphis brassicæ* Linn.; they are also the same as those used by Kelly in his work with *Aphis rumicis*.

Methods.

In order to obtain as nearly as possible uniform conditions for these experiments, I reared all of the aphids on young wheat plants. These wheat shoots were never allowed to get to be more than ten or fifteen days old, and I kept them nipped back to a height of not more than three or four inches. New plants were reared from seed for each generation of plant lice. All of the plants were kept upon a single large table in the middle of the laboratory, so that changes in heat or moisture would affect all individuals of each fraternity in much the same way.

The wheat plants used were grown in small flower pots, a single plant being raised in each pot. Soon after the aphids were born they were separated, and each nymph was placed on a single wheat plant growing in its own pot. This aphid was confined by placing a small lamp chimney, covered at the top with surgeon's gauze, over the wheat plant. This would permit ready observation of the developing aphid, and would also permit the transpiration of the wheat plant. Each individual was given a label which showed the generation to which it belonged, and also its individual number in this generation. Thus, $F_7 14$ would mean individual number fourteen of the seventh filial generation.

When it came to taking measurements of the third and fourth segments of the antennæ in order to record the same and to select individuals for carrying on the strain I encountered several difficulties. At first I tried to measure these in the live adults by weighing down the insect with a cover-glass, but this would not enable me to make accurate measurements. After trying several other methods I finally decided to let each adult remain on its own wheat plant, and produce her progeny; after which

she was killed, and the head, with the antennæ, was severed from the body, cleared in Gage's mixture, and mounted in balsam on a microscope slide. This method I found to be the only satisfactory one, for two reasons. First, measurements of the segments of the antennæ could be made accurately; and second, the fecundity of the individuals could be tested before the selection was made, a very important factor, since it frequently happens that some adults have very few or no offspring.

Having made the measurements of the antennal segments, and obtained the index for the same, I selected from each fraternity the individual or individuals desired. These were usually those whose antennal index was either the lowest or the highest in the fraternity, provided they had left a sufficient number of offspring. Now the offspring of the individuals selected were separated and placed on individual plants; those of the other individuals of the fraternity were discarded.

In making the measurements of the segments of the antennæ a mechanical eye micrometer was used. Both antennæ were measured, and an average obtained from the two measurements in order to get the index for the individual. From these indices ratios were obtained, segment four being taken as unity.

Having obtained the ratio of the length of segment three in each case to the length of segment four, I have plotted (Fig. 1) the results of the selections for ten generations. For the first six generations random selections were made in order to be sure of a pure strain and to determine the average mean index for this strain. The mean thus obtained gave a ratio of 1.80:1, that is, segment three of the antennæ was found to be one and eight tenths times as long as segment four. This ratio established for the pure line is represented by the heavy horizontal line running through about the middle of the plot in Fig. 1.

Results.

On April 9 the first individual was isolated, a wingless asexual female, and she became the mother from which all the succeeding individuals were descended. This stem mother gave birth to twenty-nine offspring. She was now killed and her antennal index taken. It was found to be 1.77:1. From the twenty-

nine offspring I selected one at random, with which to carry on the strain. The individual selected was F_13 (that is, individual number three of the first filial generation). This individual gave birth to ten young. Her antennal index proved to be 1.77:1, the same as that of her mother. From the F_2 individuals I selected at random again an individual which became the mother of six offspring. Her index was, as later obtained, 1.67:1, while that for the mean of her offspring was 1.77:1.

For the next three generations selections were made at random, a prolific or healthy looking individual being taken. The measurements of the antennal segments of all the individuals were taken, and from these the fraternal means for the second, third, fourth, and fifth generations obtained. For the first filial generation I obtained the index of the single individual used to carry on the strain. Using this index and that of the stem mother I obtained a curve for the first five generations. This I have plotted (see Fig. 1). It will be noted here that in the case of the selections of individuals representing extreme variations, as was done in the second and third filial generations, that the mean of their offspring came back to or almost to that of the strain.

In the fourth generation an individual was selected which had an antennal formula near that for the mean of its fraternity, and exactly the same as that obtained for the strain; yet its offspring gave a mean much higher than that of any individual belonging to the fraternity from which the mother was taken! (See Fig. 1.) More than this, the mean of this fraternity was higher than the index for any individual of any fraternity previously obtained. From the individuals of the F_5 generation one was obtained with a low index. It gave a brood of normal individuals.

It was from among the individuals of the F_6 generation that I began making my selections in an attempt to obtain two strains, one with a high antennal formula, the other with a low antennal formula, from this isolated pure line of parthenogenetically reproducing plant lice. F_65, with a formula of 1.88:1, was selected for obtaining a strain with a high index; F_63, formula 1.66:1, was taken for obtaining a strain with a low index. F_65

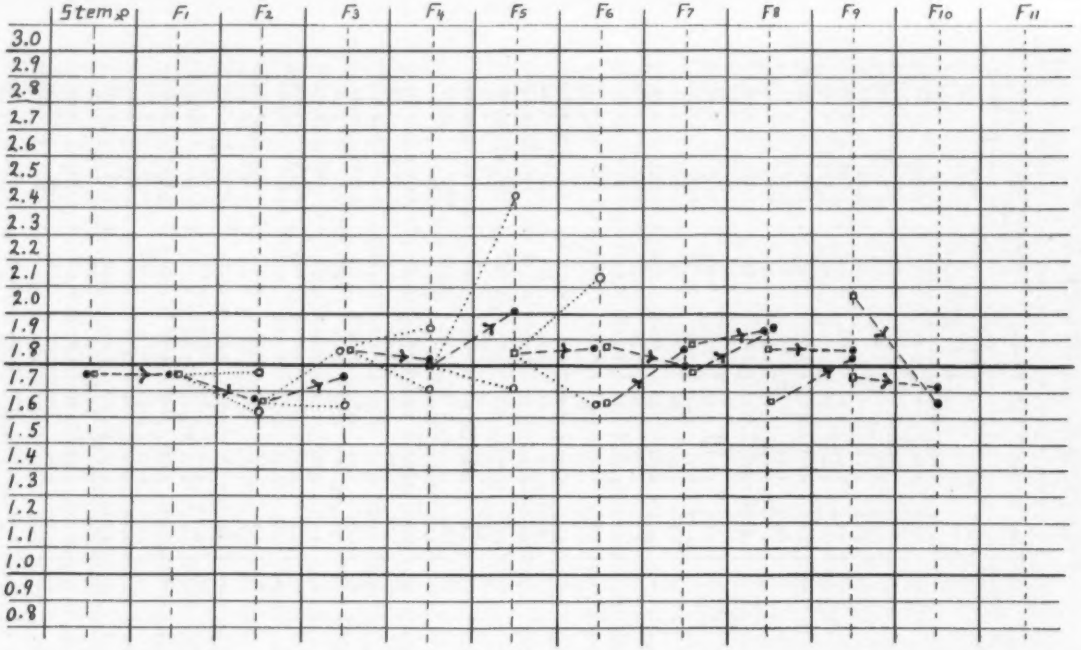

FIG. 1. Diagram showing the plotted results of selections within a pure line of *Aphis avenæ* Fab. Each black circular spot represents the position of a fraternal mean; the open circles represent the positions of the extreme variants of a fraternity; each square represents the position of the individual selected for carrying on the strain or pseudostrains. The arrows indicate the course taken by the strain as it passes from the selected individual to the mean of its offspring (the fraternity of the next generation). The heavy line at 1.8 represents the position of the mean of the strain, and may be called the mean-of-the-strain base line.

TABLE OF MEASUREMENTS AND RATIOS.

Generation and No. of Individual.	Wings.	Left Antenna.		Right Antenna.		Average for Both Antennæ.		Ratio.
		3d Jt.	4th Jt.	3d Jt.	4th Jt.	3d Jt.	4th Jt.	
Stem ♀	−	3.20	1.80	Lost	.....	3.20	1.80	1.77:1
F_{13}	−	3.70	1.80	3.70	2.25	3.70	2.025	1.82:1
F_{21}	−	3.40	1.95	3.20	2.00	3.30	1.975	1.67:1
F_{23}	−	3.35	1.80	3.15	1.90	3.25	1.85	1.78:1
F_{28}	−	3.50	2.15	Lost	.....	3.50	2.15	1.63:1
F_{31}	−	2.95	1.50	2.90	1.65	2.925	1.575	1.86:1
F_{33}	−	2.70	1.65	2.50	1.50	2.60	1.575	1.65:1
F_{35}	−	3.05	1.60	2.85	1.70	2.95	1.65	1.79:1
F_{41}	−	2.40	1.40	Broken off		2.40	1.40	1.71:1
F_{43}	−	2.35	1.25	2.15	1.25	2.25	1.25	1.80:1
F_{46}	−	Broken off		2.45	1.25	2.45	1.25	1.96:1
F_{51}	−	2.70	1.10	2.70	1.10	2.70	1.10	2.45:1
F_{52}	−	2.65	1.10	2.15	1.00	2.40	1.05	2.28:1
F_{53}	−	3.15	1.75	3.20	1.85	3.175	1.80	1.76:1
F_{56}	−	2.80	1.50	2.80	1.75	2.80	1.625	1.72:1
F_{58}	−	3.75	1.80	3.30	1.65	3.525	1.725	2.04:1
F_{510}	−	3.70	1.80	3.70	2.20	3.70	2.00	1.85:1
F_{61}	−	2.75	1.50	2.75	1.50	2.75	1.50	1.83:1
F_{63}	−	3.10	1.85	3.15	1.90	3.125	1.875	1.66:1
F_{65}	−	3.05	1.65	3.25	1.70	3.15	1.675	1.88:1
F_{66}	−	3.15	1.70	Broken off		3.15	1.70	1.85:1
F_{62}	+	Broken off		3.60	1.80	3.60	1.80	2.00:1
F_{64}	+	3.25	1.65	3.70	1.60	3.475	1.625	2.14:1
F_{73}	−	3.60	2.00	3.65	1.82	3.625	1.91	1.89:1
F_{74}	−	3.30	1.85	3.40	1.90	3.35	1.875	1.79:1
F_{72}	−	4.00	2.00	3.80	2.20	3.90	2.10	1.86:1
F_{76}	−	Lost	.....	4.15	2.10	4.15	2.10	1.98:1
F_{78}	−	3.85	2.25	Lost	.....	3.85	2.25	1.71:1
F_{711}	−	3.70	1.95	Broken off		3.70	1.95	1.90:1
F_{81}	−	4.60	2.55	4.60	2.40	4.60	2.475	1.86:1
F_{812}	−	3.55	1.75	3.50	2.10	3.525	1.925	1.83:1
F_{82}	+	4.70	2.25	4.70	2.40	4.70	2.325	2.02:1
F_{83}	+	Misplaced		4.95	2.50	4.95	2.50	1.98:1
F_{84}	+	4.30	2.20	4.70	2.45	4.50	2.325	1.93:1
F_{85}	+	3.70	1.95	3.95	2.00	3.825	1.975	1.99:1
F_{86}	+	4.20	2.05	3.75	2.00	3.975	2.02	1.91:1
F_{87}	+	4.10	2.20	4.00	2.05	4.05	2.125	1.90:1
F_{88}	+	4.00	2.00	Broken off		4.00	2.00	2.00:1
F_{810}	+	Broken off		4.75	2.40	4.75	2.40	1.98:1
F_{811}	+	4.20	2.16	3.85	2.20	4.025	2.18	1.85:1
F_{813}	−	4.20	2.30	4.15	2.30	4.175	2.30	1.81:1
F_{816}	−	3.40	1.17	3.40	1.17	3.40	1.17	2.90:1
F_{817}	−	3.80	2.25	3.75	2.15	3.775	2.20	1.71:1
F_{819}	−	3.60	2.20	3.60	2.10	3.60	2.15	1.67:1
F_{814}	+	4.10	2.30	4.10	2.30	4.10	2.30	1.78:1
F_{815}	+	4.25	2.15	Broken off		4.25	2.15	1.97:1
F_{818}	+	3.40	1.80	3.20	1.80	3.30	1.80	1.83:1
F_{917}	+	3.20	1.80	Displaced		3.20	1.80	1.77:1
F_{918}	+	3.35	1.80	3.05	1.80	3.20	1.80	1.77:1
F_{919}	+	4.00	2.20	Broken off		4.00	2.20	1.81:1
F_{920}	+	3.85	1.75	3.75	1.95	3.80	1.85	2.05:1
F_{922}	+	3.60	2.05	3.60	2.00	3.60	2.025	1.77:1
F_{91}	+	3.30	2.00	3.35	2.00	3.235	2.00	1.66:1
F_{92}	+	Broken off		3.35	1.75	3.35	1.75	1.91:1

TABLE OF MEASUREMENTS AND RATIOS (*Continued*).

Generation and No. of Individual.	Wings.	Left Antenna.		Right Antenna.		Average for Both Antennæ.		Ratio.
		3d Jt.	4th Jt.	3d Jt.	4th Jt.	3d Jt.	4th Jt.	
F_93.....	+	Broken off		Broken off		.....	.....	.....
F_94.....	+	Broken off		3.40	2.00	3.40	2.00	1.70:1
F_95.....	+	3.20	1.75	3.35	1.95	3.275	1.85	1.77:1
F_96.....	+	3.80	1.80	3.30	1.70	3.55	1.75	2.03:1
F_98.....	+	3.60	1.85	3.35	2.00	3.475	1.925	1.80:1
F_99.....	−	3.70	1.80	3.80	1.80	3.75	1.80	2.08:1
$F_{10}1$.....	+	2.90	1.80	3.10	1.75	3.00	1.775	1.70:1
$F_{10}2$.....	+	Broken off		2.80	1.75	2.80	1.75	1.60:1
$F_{10}3$.....	+	3.00	1.65	Displaced		3.00	1.65	1.82:1
$F_{10}4$.....	−	3.15	1.80	3.00	1.85	3.075	1.825	1.63:1
$F_{10}5$.....	−	2.50	1.50	2.50	1.50	2.50	1.50	1.66:1
$F_{10}6$.....	−	3.25	1.90	3.30	2.05	3.275	1.975	1.65:1
$F_{10}7$.....	−	2.50	1.60	2.50	1.60	2.50	1.60	1.56:1
$F_{10}11$.....	−	2.40	1.40	Broken off		2.40	1.40	1.71:1

gave me five young, only two of which came to maturity and gave an average index of 1.80:1. F_83 gave birth to eight individuals, four of which came to maturity, and gave as a fraternal mean an index of 1.88:1. Although the number of individuals obtained are too small to draw any conclusions, if they were not supported by a repetition of similar results, yet they are very significant, as is shown by the plot in Fig. 1. The curves running from the two extreme individuals selected of the F_8 generation, to the fraternal means of the offspring of these two individuals actually cross! In other words, offspring from an individual with a high index had a lower fraternal mean than the offspring from the individual (of the same fraternity as the former) with a low index. Among the F_7 individuals two were selected; one, F_73 with an index of 1.89:1, and one, F_74, with an index of 1.79:1. From the former, F_73, eleven young were reared to maturity. They gave a fraternal mean of 1.93:1. This is almost the same formula as was obtained for the mean of the offspring of the latter individual, F_74, which was 1.95:1. Here we get convergence almost to the same point in the offspring of two variants of a fraternity. However, both of these fraternities, that is, of the F_8 generation, did not show a reversion toward the mean of the original strain, but a deviation from it. This is what occurred in the case of the F_5 generation. In the F_8 fraternity, F_81, formula 1.86:1, and F_819, formula 1.67, were

selected, each being a descendent of a different individual of F_7 generation. $F_8$1 gave offspring with a mean of 1.85:1, and $F_8$19 offspring with a mean of 1.83:1. The former converged slightly toward the mean base line of the original strain, the latter reverted not only to it but beyond it, so that the means of the two fraternities almost meet. From these fraternities $F_9$9, formula 2.08:1, and $F_9$17, formula 1.77:1, were taken, each again being selected from the different pseudostrain. Although the former had an antennal formula very high, 2.08:1, in fact higher than that of any individual selected during the whole experiment, yet its seven offspring gave the remarkably low fraternal mean of 1.66:1. Thus reversion in this case was not to the mean of the original strain but much beyond it, as has been noted before in the case of selections from the F_6 and F_8 generations. From $F_9$17, only a single individual was obtained which gave a formula of 1.71:1, a slight deviation from the mean base line.

Conclusions.

The following conclusions can be drawn from the results obtained. Selections from among extreme variants do not alter the mean as obtained for the strain without selection. The fraternal mean of any generation may show a great fluctuation from the mean of the strain, but this fluctuation is not transmitted to following generations. The offspring of an extreme variant may show not a reversion to the mean of the line or strain, but a reversion which swings pendulum-like much beyond this mean, only to be brought back to its former side of the mean-of-the-strain base line in the next generation. This phenomenon I call oscillating reversion. Great deviations from the mean of the strain occur among fraternities which have been produced by individuals whose formula is almost the same as that of the mean of the strain. This great divergence is due to some underlying cause not yet determined, and is independent of the nature or amount of food.

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THE AXIAL GRADIENT IN CILIATE INFUSORIA.

C. M. CHILD.

FROM THE HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO.

The discovery of axial gradients in rate of metabolic reactions in *Planaria* (Child '12, '13*a*, '13*c*) and various other forms together with a considerable body of evidence, mostly as yet unpublished, which points to a close relation between such axial gradients and polarity and symmetry suggested the desirability of determining whether there is any indication of the existence of such axial gradients in those protozoa which possess a definite morphological polarity. If an axial gradient in rate of metabolic reactions is the basis of organic polarity or is in any way associated with it we must expect to find such a gradient in single cells where a definite stable polarity exists as well as in multicellular axiate organisms. The results obtained with various ciliates have fully justified this expectation. In all the forms tested thus far evidence for the existence of an axial gradient has been found.

I. METHODS.

In these experiments the method which I have called the direct susceptibility or resistance method (Child, '13*b*) was used, with KCN as the reagent in most cases. This method is based upon the fact determined by extensive experimentation, that in concentrations of the cyanides and various anesthetics which kill within at most a few hours, individuals or regions with a higher rate of metabolism or at least of the oxidation processes are more susceptible and die earlier than those with a lower rate.

In the experiments with ciliates concentrations were found which did not kill the animals at once but permitted life to continue for a period ranging from a few moments up to several hours according to the concentration. The purpose in thus limiting the concentrations used is first to avoid killing the animals too rapidly, in which case the death and disintegration of all parts may be so nearly simultaneous that a gradient if

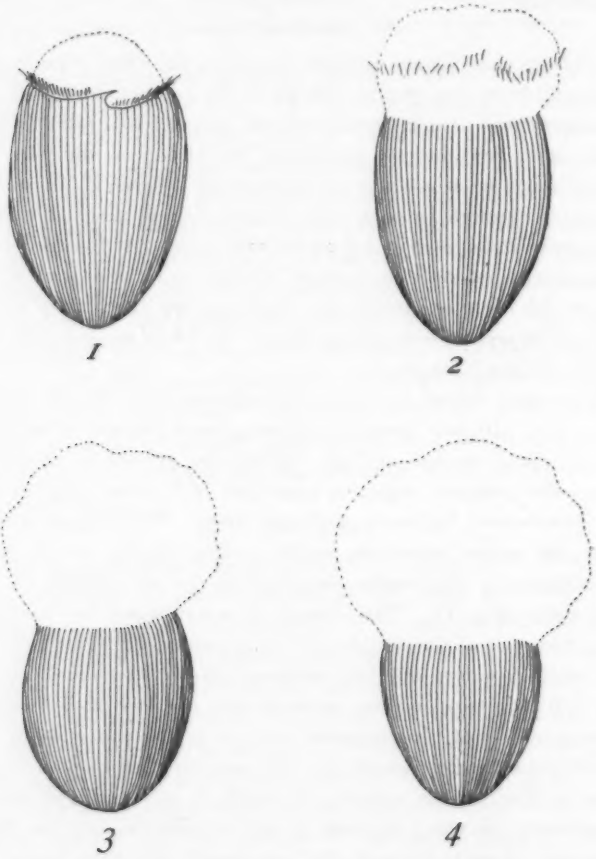
present will be scarcely or not at all apparent. If on the other hand the concentration is too low, the results may be complicated by acclimation, the individuals or regions of highest rate showing in general the greatest capacity for acclimation. The proper concentrations for each species must of course be determined by experiment.

II. Heterotricha.

The species *Stentor cæruleus* has been the chief experimental material from this group. In KCN the animals do not become elongated and attach themselves but contract to a rounded form and swim continuously backward by means of the cilia. In KCN 0.002 *m*. at a water-temperature of 25°–26° C. all animals (several hundred in each experiment) remain alive and intact and swim continuously during the first hour. At the end of this time a very few individuals show the first stages of disintegration. From this time on death and disintegration occur until at the end of the second hour all are dead. At lower temperatures the survival time is longer.

The usual course of death and disintegration is indicated in Figs. 1–5. In each figure the disintegrated portion is indicated merely by a dotted outline. While the animal is swimming about the peristome region or some part of it, often a region near the mouth first, begins to swell and bulge. This change spreads over the whole peristome within a few seconds or in many individuals is practically simultaneous in all regions of the peristome (Fig. 1). This change is accompanied by complete disappearance of the ectoplasmic striations and other structure in the region involved and the entoplasm slowly swells and spreads out. Within two or three seconds after the first indications of change a halo of blue pigment appears in the water about the disintegrated region which has now lost most of its blue color. After a short pause varying in length in different individuals from twenty or thirty seconds to two or three minutes the disintegration extends beyond the peristomal cilia and begins its course toward the aboral end of the body (Fig. 2), obliterating the morphological structure of the cytoplasm as it proceeds. The boundary between the disintegrated and intact region is always sharp and clear and the course of disintegration can be

followed without the slightest difficulty. The change at any given level is almost instantaneous: the structure seems simply to dissolve, the cytoplasm swells and the halo of blue pigment appears in the water while the disintegrated part loses most of its color. The peristomal cilia continue to beat until the instant



when the boundary of the spreading areas of disintegration reaches them when they suddenly stop in most cases, although occasionally they may beat for a few seconds more, but in all cases they disintegrate very soon. As soon as the

peristomal cilia cease their movement the body ceases to revolve in the water, but the smaller cilia covering the body continue to beat until the disintegration wave reaches them. From the peristome region the process of disintegration spreads downward over the body as indicated in Figs. 2–5, the aboral end

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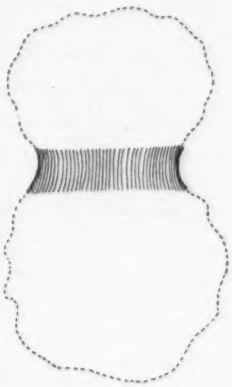

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being the last region to lose its structure. The disintegration of the aboral half is in practically all cases much more rapid than that of the oral half; very commonly the disintegration of the oral half requires two to four minutes, that of the aboral half one to two minutes, *i. e.*, the differences in susceptibility beween different levels are much greater in the anterior than in the posterior half. Moreover, the anterior half and particularly the peristome region lose their pigment more completely and swell and spread out to a greater extent in disintegration than the posterior half. Finally the animal is represented by a shapeless mass of protoplasm in which the meganucleus remains intact for a long time and about which a halo of blue pigment appears. With the concentration and temperature above mentioned the whole process of death and disintegration requires from two to six minutes, the rate of advance of the disintegration varying in different individuals and in some cases in different regions of the body.

The striking features of the change are its very definite course,

the distinct boundary between disintegrated and intact regions at all times and the uniformity with which the change advances at all points of the circumference of the body. Occasionally, however, modifications of the process appear. In a small percentage of the animals used for the experiment, not more than one or two per cent., the advance of disintegration ceases for a time when it has reached about the stage represented in Fig. 3, *i. e.*, when about the oral half is disintegrated. Then after one or two minutes the disintegration of the aboral half occurs almost simultaneously in all parts. In one individual observed (Fig. 6) the disintegration of the oral half occurred in the usual manner, then there was a pause of about one minute and then the disintegration of the aboral half began from the aboral end. Fig. 6 shows this individual a few seconds before disintegration was complete. A narrow band of intact body wall on which the cilia were seen to be still moving separates the two areas of disintegration. During the next few seconds the aboral area advanced over this band and completed the process. This is the only case among thousands observed in which disintegration proceeded from the aboral end instead of toward it.

The length of life in the reagent before disintegration begins and the rate of advance of disintegration over the body vary within wide limits according to water-temperature, concentration of the reagent and alkalinity, but the course of the process is the same in all cases with the rare modifications described above.

Another species of *Stentor*, apparently *S. polymorphus*, gives the same results, but the course of disintegration is not so readily followed in this species because of the absence of pigment and the less conspicuous myonemes.

III. Hypotricha.

Several species of hypotrichous ciliates including two species of *Stylonychia*, *Euplotes* and two unidentified forms have thus far been tested by this method and with the same result in all cases, viz., death and disintegration begin at the anterior end and proceed down over the body to the posterior end. When the animals are placed in KCN they at once begin to swim backward and continue to do so until death and disintegration occur.

The process of disintegration is in all essential features similar to that in *Stentor* and consists in a dissolution of the structure and a swelling of the cytoplasm. The boundary between the disintegrated and intact regions is sharp and the disintegrative change proceeds uniformly down the body, the rate of advance varying in different individuals and with different temperatures and concentrations of reagent.

In *Stylonychia* the beginning of disintegration is marked by the melting down of the structures at the anterior end into a mass of uniform semi-fluid cytoplasm while the cilia elsewhere are still active and the animal is moving rapidly backward. The dissolution of structure extends backward over the body and the disintegrated mass often stretches out and is left behind in a long trail of granules and semifluid droplets as the animal moves backward. Movement continues in each part until the advancing wave of disintegration reaches it, when it suddenly breaks down and seems to dissolve into a formless mass. The posterior end continues to move until finally it too dissolves and movement ceases.

The advance of the disintegration wave and the sudden melting of each structure as the wave reaches it are striking phenomena and the uniformity of the whole process in different individuals and in different species indicates that it is associated with a fundamental feature of the constitution of these cells.

If the concentration of the cyanide is so low that the animals live for an hour or more before disintegration begins *Stylonychia* usually loses its flattened elongated form and becomes almost spherical, but movement still continues and the anterior end is still distinguishable by its structure. In such cases the disintegration, when it finally occurs, is more rapid and extends from anterior to posterior end in a few seconds.

IV. Peritricha.

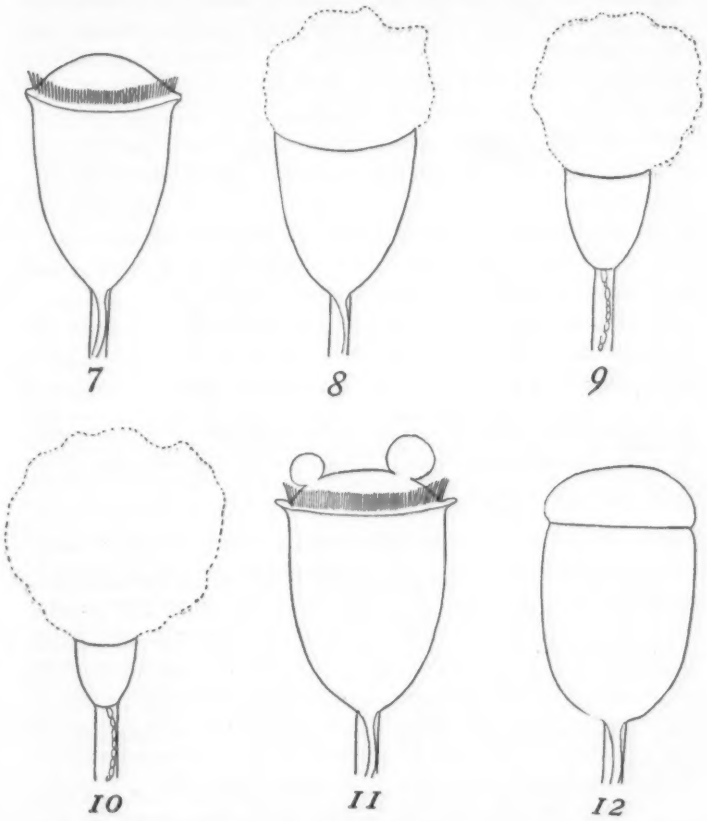
The forms examined include three species of Vorticellidæ and one of *Carchesium*. The results are practically identical in all cases. All of these forms are more resistant than *Stentor* and require a higher concentration of reagent. In *Carchesium* for example the course of death and disintegration in KCN

0.01 *m.* is as follows: The first change is a bulging of the peristome (Fig. 7) as in *Stentor*, but in these forms this does not usually occur until after the cilia have ceased or almost ceased to beat. This bulging is followed within a few seconds by disintegration of the peristome region including the cilia (Fig. 8), while the rest of the body is still intact and then a pause of a few seconds occurs. Disintegration of the body below the peristome is much more rapid than in *Stentor* and during the next few seconds the structure, except for the meganucleus, disappears completely and the entoplasm swells and spreads out into the water, the process beginning at the oral region and rapidly advancing aborally. But the ectoplasm does not disintegrate completely: a part of the body wall is elastic and as the contents of the body flow out this structure contracts and remains visible for a long time as a transparent skeleton or "shadow" of the body, much reduced in size but retaining more or less the original shape (Figs. 9 and 10). So far as can be determined the ectoplasm of the peristome region does not take part in the contraction but after the early stages of disintegration disappears completely. Evidently the lateral body wall is differentiated in part into a supporting substance which is no longer metabolically active. In this connection the difference in contractility between the peristome region and the lateral walls of the body in the living animal is of interest. The peristome is highly contractile and can be closed and opened while other parts of the body wall are capable of but little contraction. The contractile region disintegrates completely at once in KCN while the non-contractile region does not.

The stalk resembles the lateral body wall in its resistance but the contractile fiber usually shows disintegrative changes at about the same time as the peristome region or a little later. These changes consist in a loss of the regular contour, the appearance of varicosities and an apparent breaking up into a series of granules or droplets. Examination of the fiber with very high powers has not been made.

When lower concentrations, *e. g.*, KCN 0.002 *m.* are used, the death changes are limited to the appearance of hyaline or slightly granular droplets on the peristome region (Fig. 11) or to a bulging

of the whole peristome (Fig. 12) and the body may remain otherwise intact for several days. The changes in ethyl ether 2 per cent. are very similar. In a small vorticellid of which only a few individuals were present in my cultures the death changes consisted of the bulging of the peristome (Fig. 13) and later of



the disappearance of the cilia and the swelling of the cytoplasm until it formed a large rounded mass still enclosed within the stretched membrane of the peristome region (Fig. 14) while the lateral body-wall retained its form. In higher concentrations the changes in this form would undoubtedly be the same as in *Carchesium*.

In all four species examined the peristome region is very evidently more susceptible to the reagent than other parts. In the remainder of the body the death changes do progress from the oral to the aboral end, but their progress is much more rapid than in *Stentor*. In KCN 0.01 *m*. disintegration is completed within 5–10 seconds after the pause following peristome disintegration. In these forms then the axial gradient consists first

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in a marked decrease in susceptibility from the peristome region to more aboral regions and a slight decrease in the rest of the body from the oral to the aboral end.

V. Holotricha.

Paramœcium is not very susceptible to KCN, but the individual differences in resistance are considerable. In KCN 0.01 *m*. with water-temperature 22°–25° C. average survival time of animals from one culture was six or seven hours, but some animals die after two or three hours and some live eight or ten hours. Moreover, different cultures differ very considerably in resistance to cyanide.

When the animals are placed in KCN they at once begin to swim backward. This continues for a period varying from a few minutes to half an hour or in some cases longer. Gradually the backward movement becomes less and less marked until the animal is simply revolving and then sooner or later it begins to swim forward once more and continues until it dies.

In KCN 0.01 *m*. the disintegration changes begin in the great

majority of cases with the appearance of a rounded swelling over the anterior vacuole. The ectoplasm involved loses its cilia and its characteristic structure but a thin membrane remains surrounding the swelling. The swelling gradually increases in size and becomes a spherical mass into which the anterior vacuole often passes (Fig. 15). Here the vacuole undergoes enlargement and does not contract rhythmically but finally disappears. The vacuole is surrounded by granular cytoplasm. In other cases the anterior vacuole remains in its normal position and the swelling contains only granular cytoplasm (Fig. 16). In all cases the swelling is lighter in color than other parts of the body and the characteristic structure and the cilia of the region have disappeared.

Occasionally the swelling appears over the posterior instead of the anterior vacuole (Fig. 17) but this is comparatively a rare occurrence. In a few individuals among the thousands observed swellings of about equal size appeared over both vacuoles (Fig. 18) and in a few other cases a larger swelling over the anterior, a smaller over the posterior vacuole. But in at least nine out of ten the swelling appeared only on the anterior vacuole.

The swelling gradually enlarges, while the animal continues to swim, the vacuole disappears sooner or later (Fig. 19) and the intact portion of the body gradually decreases in size, the ectoplasm evidently undergoing contraction as the entoplasm gradually passes into the swelling (Fig. 20). At this stage the animal is still swimming, the structure of the ectoplasmic regions not involved in the swelling is intact and meganucleus and posterior vacuole retain their positions. As noted above the cytoplasm in the swelling, which is now as large as the remainder of the body, is lighter in color and more transparent and more uniform in appearance than in the intact regions. It is evidently dead and some of its constituents have probably diffused through the membrane as does the blue pigment in the case of *Stentor*.

The disintegrative change gradually spreads over the ectoplasm, less rapidly on the side of the oral groove than on the opposite side, and the intact ectoplasm continues to decrease in size (Fig. 21). The cilia on the intact portion are still present and beating at this stage and the animal may move slowly in a circular course with

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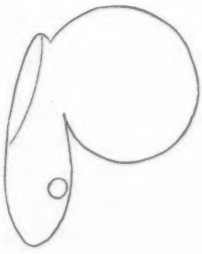

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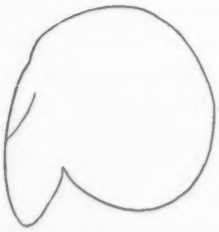

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the swelling always toward the center. This direction of movement results from the distribution of the cilia and the weight of the swelling. In many cases the swelling bursts sooner or later, the contained cytoplasm flows out, a wave of disintegration accompanied by an increase in translucency passes rapidly over the remaining portions of the body, toward the posterior end, blotting out the ectoplasmic structure and the cilia and all that remains is the mass of granules spreading in the water, the meganucleus and a small transparent ectoplasmic "shadow" of the portions not directly involved in the swelling (Fig. 22).

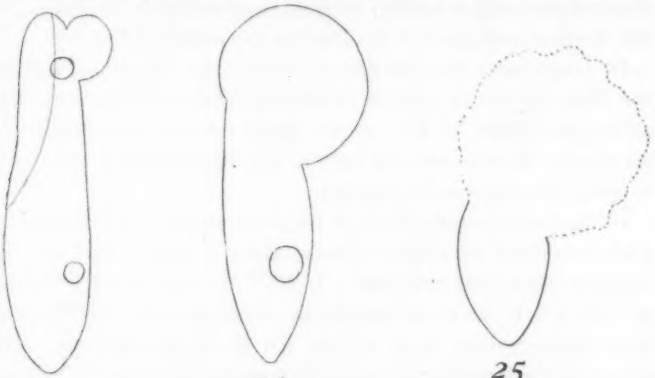
In other cases the dissolution of structure occurs throughout the body before the rupture of the membrane and the body may retain the shape of Fig. 21 for some minutes but finally the membrane disappears and only a shapeless granular mass containing the meganucleus remains.

In higher concentrations of KCN or in lower concentrations with increased alkalinity disintegration is more rapid but its course is essentially the same. In KCN 0.02 *m*. or in KCN 0.001 *m*. which is made more alkaline by the addition of NaOH death and disintegration occur within ten to fifteen minutes. The course of disintegration is much the same as that described above, a small swelling containing granular cytoplasm lighter in color than the rest appears over the anterior vacuole, but the vacuole does not enter it in most cases (Fig. 23); this swelling increases rapidly in size and soon involves the anterior end of the body (Fig. 24); sooner or later its membrane disappears, the wave of disintegration spreads over the body, blotting out its structure and only a mass of granular protoplasm and the contracted "shadow" remaining (Fig. 25). In a few individuals droplets of semi-fluid hyaline substance appeared on other parts of the body surface and increased in size until bursting occurred.

In these solutions a swelling over the posterior vacuole appears much more frequently than in KCN 0.001 *m*. without the addition of alkali or even in 0.01 *m*. In most cases where a posterior swelling appears it is smaller than the anterior, but occasionally it is larger and rarely only the posterior swelling appears. In general, the higher the concentration of the reagent, whether with or without the addition of alkali, the more frequently the

posterior as well as the anterior vacuole region swells. Increase in alkalinity accomplishes the same result as increase in concentration of KCN, *i. e.*, it increases susceptibility. In other words, when the concentration is so low that the animals die very slowly only the anterior vacuole region undergoes swelling before the death and disintegration of the rest of the body.

In still higher concentrations, *e. g.*, KCN 0.04 *m.* the animals die very soon, hyaline droplets appear on all parts of the body



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and in a few seconds nothing remains but the entoplasmic granules and the meganucleus more or less surrounded by the semi-fluid hyaline substance in rounded masses or droplets, apparently with a surface membrane. Sooner or later the droplets burst, apparently in consequence of rupture of the membrane, and the substance of the body spreads through the water. In such cases a gradient does not usually appear because the concentration is sufficiently high to kill all parts at the same time or nearly so.

Discussion.

It is evident from the preceding sections that a more or less definite axial gradient in susceptibility to KCN exists in all of the forms examined. These forms represent the four orders of ciliate infusoria, Heterotricha (*Stentor*), Hypotricha (*Stylonychia*, *Euplotes*, etc.), Peritricha (*Vorticella* and *Carchesium*) and Holotricha (*Paramœcium*), so that the existence of an axial gradient is evidently characteristic of the group.

If the susceptibility is in any degree a measure of the rate of metabolism or more specifically of oxidation processes as it unquestionably is in many other cases (Child 13*b*) we must conclude that an axial gradient in the rate of these processes is a characteristic feature of the ciliates. Moreover, the structure and behavior of the animals also suggest differences in rate of metabolic processes along the axis. In *Stentor* the peristome region is undoubtedly the most sensitive, most highly contractile and most active region of the body. In *Vorticella* and *Carchesium* a similar difference exists between peristome and body exclusive of the contractile fiber of the stalk. In *Stylonychia* the development and activity of cilia are greater in the anterior region than elsewhere. In the case of *Paramœcium*, however, the structure and behavior do not afford any definite evidence upon this point.

In forms like *Stentor*, *Vorticella* and *Carchesium*, where a special contractile peristome region is present, this region is distinguished from other regions of the body by a considerably greater degree of susceptibility, while in *Stylonychia* the gradient of susceptibility is almost uniform. Moreover in the elongated body of *Stentor* the gradient is steeper in the anterior than in the posterior half, *i. e.*, in the region which forms the stalk the gradient is relatively slight as compared with the body proper. In short there can, I think, be no doubt that the differences in susceptibility along the axis in *Stentor*, *Stylonychia*, *Euplotes*, *Vorticella* and *Carchesium* are an expression of a dynamic axial gradient, a gradient in the rate of certain fundamental metabolic processes.

The case of *Paramœcium* requires some further consideration. The constant association of the swelling with the vacuoles, and in the great majority of cases with the anterior vacuole alone raises the question whether we are to regard this region as possessing a higher rate of metabolism or of respiration than other parts of the body or whether some complicating factor, *e. g.*, weakness of the ectoplasmic wall in this region, plays a part in the localization of the swelling. There is no visible indication that the ectoplasm of the vacuolar region is thinner or structurally different in any way from that of other regions of the body, but such negative evidence is of course not conclusive. On the other

hand the fact that the vacuole remains in its normal position at least as often as it enters the swelling indicates that the swelling is not simply the result of the enlargement of the vacuole and consequently stretching of the body wall. It should also be noted that when the swelling appears the ectoplasm does not merely stretch, it loses its structural features, including the cilia and the entoplasm which the swelling contains differs in appearance from that in other parts of the body. And finally, the vacuolar mechanism is very sensitive to cyanide, the pulsation ceases soon after the animal is placed in the solution and the vacuole slowly undergoes enlargement and sooner or later collapses and disappears. The vacuole region is apparently a specialized contractile mechanism and is undoubtedly the seat of a relatively high rate of dynamic activity. In short the only logical conclusion is that the greater susceptibility of the region where the swelling appears results from greater dynamic activity.

But a difference of some sort must exist between anterior and posterior vacuolar regions, for it was pointed out above (p. 47) that in the lower concentrations of the reagent only the anterior vacuolar region shows a swelling and that the frequency of swelling in the posterior as well as in the anterior vacuolar region increases as the concentration increases. Evidently the posterior vacuolar region is for some reason less susceptible than the anterior and a higher concentration is necessary at least in the great majority of individuals, to bring about swelling in it than in the anterior region.

A comparison of the rate of pulsation in anterior and posterior vacuoles affords a clue to the nature of this difference. I have found that in general the rate of the anterior vacuole is considerably higher than that of the posterior. Sometimes the ratio is as high as 5 : 3, in other cases less, but the difference in rate exists, at least in the great majority of individuals. Evidently the posterior vacuole has a lower rate of dynamic activity than the anterior, and the difference in susceptibility of the two vacuolar regions is undoubtedly associated with this difference in rate. In the lower concentrations or in individuals with a low susceptibility the special susceptibility appears as a rule only in the anterior vacuolar region because only here is

the rate of reaction sufficiently high to bring about the rapid change. In higher concentrations the special susceptibility may appear in both vacuolar regions, but even in such cases the swelling usually appears earlier or is of larger size in the anterior region. In short the facts indicate that the two vacuolar regions of *Paramœcium* are more or less definitely localized regions of relatively high rate of metabolism or more specifically of respiration and, furthermore, that the rate in the anterior vacuolar region is higher than that in the posterior.

The lower susceptibility of the posterior vacuolar region to KCN and the lower rate of pulsation of the posterior vacuole in normal living animals suggest the existence of an axial gradient in rate of metabolism, and in the death and disintegration of the body in general this gradient appears as has been shown. When the disintegrative change begins to spread from the vacuolar region the anterior end of the body is attacked first and from this end the process of disintegration spreads downward to the posterior end. The differences in susceptibility along the axis are not as great as the differences between peristome and other regions in *Stentor* and *Vorticella*, in other words the axial gradient in rate is less steep in *Paramœcium* than in those forms.

A general relation seems to exist between the character of the gradient and the degree of morphological and physiological "cephalization" in these different forms. In *Paramœcium* the gradient is comparatively slight and apparently uniform except for the vacuolar regions. In *Stylonychia* and *Euplotes* the gradient is steeper, *i. e.*, the differences are greater but here also it is uniform or nearly so. In *Stentor* a distinct gradient, steeper in the more anterior regions than that in *Paramœcium*, extends throughout the length of the body, but the peristome region is distinctly marked off from other parts by its higher rate, *i. e.*, the gradient from the peristome region to other parts is steeper than the gradient in other regions. And finally in *Vorticella* and *Carchesium* the gradient between peristome and other regions is steep as in *Stentor* but in other parts of the body the gradient is slight. When we compare these facts with the differences in morphology and behavior of these different species and of the different regions of the body in each the general parallelism

between the different groups of facts is strikingly evident. The degree of morphological and physiological "cephalization" in these infusoria parallels the degree of difference between the rate of metabolism in the apical region and that in other regions. The obvious inference from the facts is that the axial metabolic gradient has some very intimate relation to the morphological and physiological polarity of these organisms.

The occasional departures from the general rule such as the case observed in *Stentor* where disintegration in the posterior half of the body proceeded from the posterior end and the rare cases in *Paramœcium* where a swelling appears over the posterior vacuole only undoubtedly result from temporary stimulation of the posterior regions. The dynamic gradient is not to be conceived as fixed and invariable; it may be temporarily or in many cases even permanently eliminated. But wherever it is present to a certain degree and more or less continuously existent it must determine both morphological and physiological differences along the axis.

The facts of embryology indicate that a gradient in rate of metabolism exists or arises early in the embryonic development of every animal and that the region of highest rate becomes the apical or cephalic region. In plants likewise the apical region of the axis is undoubtedly the region of highest metabolic rate.

Attention has already been called in earlier papers (Child, '13*a*, '13*c*) to these and to other facts and further data will be presented in the future. The facts indicate as I believe that a gradient in the rate of metabolism or of certain metabolic processes is the dynamic basis of morphological and physiological polarity in organisms. The existence of such a gradient in unicellular organisms and the striking relation between its features and the morphological and physiological features of the different species in these as well as in multicellular forms is to say the least suggestive. It points very clearly to a fundamental similarity between unicellular and multicellular organization and differentiation.

These observations are also of some interest in another connection. Loeb ('99) has advanced the hypothesis that the nucleus is the chief organ of oxidation in the cell and has inter-

preted the inability of non-nucleated pieces or cells to regenerate to asphyxiation in the absence of the nucleus. Verworn ('91, '04), on the other hand, has shown that non-nucleated pieces of various ciliates are as susceptible to lack of oxygen and recover as readily when it is present as nucleated cells or pieces. These facts indicate that respiration takes place in the cytoplasm, even when the nucleus is absent. Later investigations by various authors support and confirm Verworn's view.

My own observations show that the nucleus is much less susceptible to cyanide than the cytoplasm. If the cyanides inhibit in some way the oxidation processes as most authorities believe the much greater susceptibility of the cytoplasm indicates that the oxidations are localized in it, at least to a much greater extent than in the nucleus. Moreover, the existence of a gradient in susceptibility to cyanide in the cytoplasm and particularly in the ectoplasm indicates that different rates of oxidation are localized in different regions of the unicellular body in very much the same way as in multicellular forms. But even if the cyanides produce their effect in some other way than by inhibiting the oxidation processes specifically, whether their action is upon some other reactions of the metabolic complex or upon the metabolic substratum of the cell, they make it evident that a dynamic gradient exists along the main axis of the unicellular ciliate infusorian body.

Summary.

1. An axial gradient in susceptibility to KCN is present in *Stentor*, *Stylonychia*, *Vorticella*, *Carchesium* and *Paramœcium*. This suceptibility gradient indicates the existence of a gradient in rate of metabolism or of oxidation processes.

2. In all forms examined the anterior or apical region is the highest point in the general gradient, although localized regions of still higher rate such as the vacuolar regions of *Paramœcium* may exist.

3. A close parallelism exists between the character of the gradient and the morphological and physiological features of the species. Where the gradient is uniform and slight, structural and physiological differences along the axis are slight and where

the changes in steepness are great, marked morphological and physiological differences are present.

4. The facts indicate that the axial gradient is the dynamic basis of morphological and physiological polarity in the ciliate infusoria as well as in other organisms.

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